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Endocrine cells in hepatobiliary cystadenomas and cystadenocarcinomas

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Abstract We investigated the distribution of endocrine cells in hepatobiliary cystadenoma (n=5, two associated with mesenchymal stroma) and cystadenocarcinoma (n=3) immunohistochemically. In normal livers (n=20) and livers affected by hepatolithiasis (n=15) used as controls, endocrine cells revealed by chromogranin immunostaining were located exclusively in normal or proliferating intrahepatic peribiliary glands. In the eight cases of hepatobiliary cystadenoma and cystadenocarcinoma, endocrine cells were present in four cases (50%) (1 cystadenoma, 1 cystadenoma with mesenchymal stroma, and 2 cystadenocarcinomas). Endocrine cells tended to be located beneath and among the columnar epithelial cells. Intrahepatic peribiliary glands were located in the vicinity of cystadenoma or cystadenocarcinoma in six (75%) of the eight cases, and they frequently showed cystic dilatation and contained endocrine cells. Intrahepatic peribiliary glands were located in the vicinity of the endocrine cells in all cystadenomas and cystadenocarcinomas that were positive for endocrine cells. These data show that about 50% of hepatobiliary cystadenomas and cystadenocarcinomas contain endocrine cells and suggest that hepatobiliary cystadenoma and cystadenocarcinoma may originate from intrahepatic peribiliary glands.

Key words Hepatobiliary cystadenoma · Hepatobiliary cystadenocarcinoma · Intrahepatic peribiliary glands · Chromogranin

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Introduction

Hepatobiliary cystadenoma and cystadenocarcinoma are rare primary liver neoplasms characterized by cystic neoplasms lined with benign or malignant columnar epithelium of the biliary cell type [3, 4, 6, 10, 14, 18, 20]. Hepatobiliary cystadenocarcinoma usually arises from hepatobiliary cystadenoma [20]. These tumours sometimes have an ovarian-like mesenchymal stroma [20] and do not communicate with the intrahepatic biliary tree. There have been about 100 reported cases in the English literature [3, 4, 6, 10, 14, 18, 20]. Their histogenesis remains obscure.

The human intrahepatic biliary tree consists of bile ducts of different calibre with varying morphology and function. The intrahepatic biliary tree is classified into large bile ducts, septal bile ducts, interlobular bile ducts and bile ductules [8], and adnexal glandular structures (intrahepatic peribiliary glands) are present around the large bile ducts [15, 16]. Large bile ducts (luminal diameter >500 μm) are grossly recognizable and correspond to the hepatic, segmental and area ducts of Healey and Schroy [5]. Septal bile ducts (luminal diameter=100–500 μm) are small bile ducts that are not recognizable grossly and consist of columnar bile duct epithelia and outer fibrous bands. Interlobular bile ducts (luminal diameter=50–100 μm) consist of cuboidal biliary epithelia not invested with a fibrous band, and are accompanied by hepatic arteries. Bile ductules (luminal diameter <50 μm) are very small ductules not accompanied by hepatic arteries. The intrahepatic peribiliary glands are seromucous tubuloalveolar glands located around the large bile ducts [15, 16].

Endocrine cells have rarely been sought in the human intrahepatic biliary tree [7, 13]. One report suggested that they were located exclusively in the intrahepatic peribiliary glands in normal livers [7], and another, that endocrine features might be present in bile ductules in cholestatic liver diseases [13]. We have recently encountered a case of hepatobiliary cystadenoma in which many endocrine cells were present under and among the epi-

thelial lining cells; intrahepatic peribiliary glands were situated in their vicinity. This case prompted us to survey the endocrine cells and peribiliary glands in hepatobiliary cystadenoma and cystadenocarcinoma

Materials and methods

Eight hepatobiliary cystadenomas and cystadenocarcinomas were retrieved from the files of our laboratory and affilitated hospitals (Table 1). They comprised hepatobiliary cystadenomas (n=3), hepatobiliary cystadenomas with mesenchymal stroma (n=2) and hepatobiliary cystadenocarcinomas (n=3). Several tissue specimens including the cystic lesions were obtained from each case, and were fixed in 4% formaldehyde solution and embedded in paraffin. As controls, we retrieved 20 normal human livers from the recent autopsy file at our laboratory and 15 livers affected by hepatolithiasis from surgical files at our laboratory and affiliated hospitals. One to three tissue specimens were obtained from the hilar area in each normal liver and from the stone-containing bile ducts in each liver with hepatoliths. These specimens were fixed in 4% formaldehyde solution and embedded in paraffin. Two 3-μm sections were obtained from each paraffin block. One of them was stained with haematoxylin and eosin, and the other was subjected to immunostaining for chromogranin.

Endocrine cells were detected immunohistochemically by the standard avidin–biotin-peroxidase complex (ABC) method, using a pan-neuroendocrine marker, chromogranin. In brief, deparaffinized sections were incubated for 20 min in absolute methanol containing 0.3% H_2O_2 to eliminate endogenous peroxidase activity. The sections were then treated for 20 min with normal serum, followed by treatment at 4° overnight with anti-chromogranin monoclonal antibody (clone LK2H10; mouse IgG1 class; prediluted; Immunotech, Marseille, France). This antibody reacts with a 68-kDa protein that is associated with endocrine secretory granules and is considered to detect the majority of endocrine cells [21]. The sections were then treated for 1 h with biotinylated anti-mouse IgG (Vector Lab., Birlingame, Calif., USA), followed by treatment for 1 h with ABC (Vectastein ABC Kit, Vector Lab.). Reaction products were developed by 0.02% 3,3′-diaminobenzidine tetrahydrochloride solution containing 0.03% H_2O_2. Nuclei were lightly counterstained with haematoxylin. Sections of the normal pancreas and stomach were used as positive controls in each immunohistochemical run. No staining was obtained when non-immune serum or phosphate-buffered saline was used instead of the primary antibody.

Table 1 Clinicopathological findings in hepatobiliary cystadenoma and cystadenocarcinoma (*M* male, *F* female, – absent, + sparse peribiliary glands or endocrine cells, ++ moderate amount or number of peribiliary glands or endocrine cells, +++ broad or numerous peribiliary glands or endocrine cells)

Case no.	Age	Sex	Neighbouring peribiliary glands	Endocrine cells
Hepatobiliary cystadenoma				
1.	52	F	–	–
2.	54	F	+	–
3.	66	M	+++	+++
Hepatobiliary cystadenoma with mesenchymal stroma				
4.	53	F	++	+++
5.	69	F	–	–
Hepatobiliary cystadenocarcinoma				
6.	57	M	++	+
7.	67	M	++	+
8.	73	M	+	–

Results

The results are summarized in Table 1. Chromogranin-positive endocrine cells were found under and among the epithelial cells of one (33%) of the three cases of cystadenoma (Fig. 1), and in one (50%) of the two cases of cystadenoma with mesenchymal stroma (Fig. 2). The three cases of cystadenocarcinoma were composed of both cystadenoma and cystadenocarcinomatous elements. Endocrine cells were found among the crystadenocarcinoma cells and/or cystadenoma cells in two (67) of the three cases of cystadenocarcinoma (Fig. 3).

Intrahepatic peribiliary glands were present in the vicinity of cystadenoma and cystadenocarcinoma in six (75%) of the eight cases (Fig. 1, Table 1). The peribiliary glands occasionally contained endocrine cells, and showed various degrees of cystic dilatation (Fig. 1). There was a gradual transition between the dilated peribiliary glands and cystadenoma or cystadenocarcinoma. In all the cystadenomas and cystadenocarcinomas that were positive for endocrine cells, the intrahepatic peribiliary glands were present consistently in the vicinity of the cystic neoplasms.

Of the 20 normal livers, chromogranin-positive endocrine cells were found in 4 (20%), and all of them situated in the peribiliary glands (Fig. 4). Bile ducts and hepatocytes were consistently negative for chromogranin-positive endocrine cells. Among the 15 hepatolithiatic livers, chromogranin-positive endocrine cells were present in 12 (80%), with the great majority in the proliferated peribiliary glands (Fig. 5), although a minority were seen in the proliferating surface epithelium of large and septal bile ducts. Interlobular bile ducts, bile ductules and hepatocytes were consistently negative for chromogranin.

Discussion

The histogenesis of hepatobiliary cystadenoma and cystadenocarcinoma is obscure, although an origin from the biliary epithelium has been assumed. Some authors speculate that they develop from congenital remnants of sequestered foregut [6, 20]. Subramony et al. [14] have suggested an origin from ectopic rests of embryonic gallbladder tissue. Hepatobiliary cystadenocarcinoma is generally thought to arise from pre-existing cystadenoma, because cystadenocarcinoma contains areas of cystadenoma in the same sample [6, 20]. Our cases of hepatobiliary cystadenocarcinoma also appeared to arise from pre-existing cystadenoma.

The presence of endocrine cells in hepatobiliary cystadenoma was first described in 1992 by Gourley et al. [4], who detected chromogranin-positive endocrine cells in one of two cases of hepatobiliary cystadenoma with mesenchymal stroma. Subramony et al. [14] did not find chromogranin-positive endocrine cells in five cases of hepatobiliary cystadenoma. Recently, Devaney et al. [3] reported that chromogranin-positive endocrine cells were present in 6 (33%) of 18 cases of hepatobiliary cystadenoma and in 1 (10%) of 10 cases of hepatobiliary cystade-

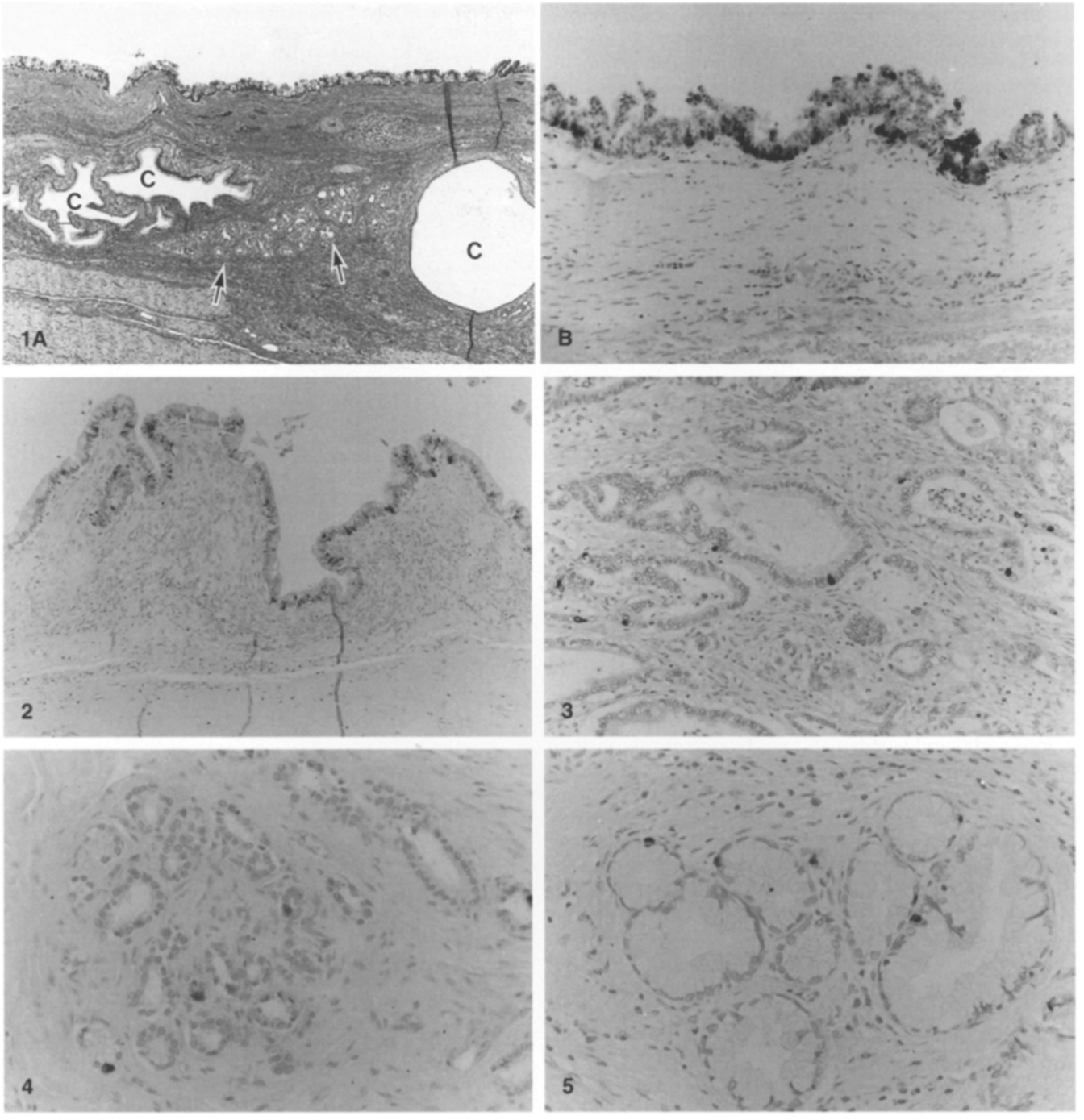

Fig. 1 **A** Low-power (*upper*) view of hepatobiliary cystadenoma. Intrahepatic peribiliary glands (*arrows*) are located in the vicinity of the cystadenoma (*above*). Some peribiliary glands show cystic dilatation (*C*). Haematoxylin and eosin, ×40. **B** Chromogranin-positive endocrine cells are present under and among the epithelial cells of the hepatobiliary cystadenoma. Immunostaining for chromogranin, ×200

Fig. 2 Chromogranin-positive endocrine cells are present under and within the epithelium of a hepatobiliary cystadenoma with mesenchymal stroma. Immunostaining for chromogranin, ×150

Fig. 3 Chromogranin-positive endocrine cells are present under and within the epithelium of hepatobiliary cystadenocarcinoma. Immunostaining for chromogranin. ×180

Fig. 4 Chromogranin-positive endocrine cells are scattered under and within the epithelium of normal intrahepatic peribiliary glands. Immunostaining for chromogranin, ×200

Fig. 5 Chromogranin-positive endocrine cells are scattered under and within the epithelium of proliferating intrahepatic peribiliary glands in hepatolithiasis. Immunostaining for chromogranin. ×200

nocarcinoma. The present study showed that chromogranin-positive endocrine cells were found in two (40%) of five hepatobiliary cystadenomas and in two (67%) of three hepatobiliary cystadenocarcinomas, thus confirming that hepatobiliary cystadenoma and cystadenocarcinoma may contain chromogranin-positive endocrine cells.

The present study revealed that intrahepatic peribiliary glands were present in the vicinity of hepatobiliary cystadenoma or cystadenocarcinoma in six (75%) of the eight cases. The intrahepatic peribiliary glands frequently showed cystic dilatation, and there was a gradual transition between the dilated peribiliary glands and cystadenoma or cystadenocarcinoma. This finding suggests that there may be a close relationship between intrahepatic peribiliary glands and hepatobiliary cystadenoma or cystadenocarcinoma.

The present study revealed that chromogranin-positive endocrine cells were present in four (50%) of eight cases of hepatobiliary cystadenoma and cystadenocarcinoma. It also revealed that chromogranin-positive endocrine cells were localized exclusively in the normal and proliferating intrahepatic peribiliary glands in normal livers and livers affected by hepatolithiasis, suggesting that among intrahepatic bile ducts the intrahepatic peribiliary glands are the only elements that contain endocrine cells. The intrahepatic peribiliary glands in the vicinity of hepatobiliary cystadenoma and cystadenocarcinoma frequently contained chromogranin-positive endocrine cells. In addition, hepatobiliary cystadenoma and cystadenocarcinoma positive for endocrine cells consistently harboured intrahepatic peribiliary glands in their vicinity. It has also been reported that the immunophenotypes of the epithelium of hepatobiliary cystadenoma and adenocarcinoma were similar to those of intrahepatic peribiliary glands [17]. These findings, together with the close association of peribiliary glands with hepatobiliary cystic neoplasms, suggest that hepatobiliary cystic neoplasms that are positive for endocrine cells may arise from the intrahepatic peribiliary glands, probably through cystic dilatation followed by neoplastic transformation. In this respect, it is noteworthy that Bhathal et al. [1] have recently demonstrated that the so-called bile duct adenoma may be derived from intrahepatic peribiliary glands (peribiliary gland hamartoma).

However, endocrine cells may be present in tumours that appear to originate from ductal epithelia, such as ampullary carcinoma, pancreatic ductal carcinoma and intraductal papillary-mucinous neoplasm of the pancreas [9, 12]. Therefore, it seems possible that the endocrine cells in the hepatobiliary cystadenoma and cystadenocarcinoma may be a phenomenon of endocrine differentiation of tumour cells, as this change is occasionally found in tumours of the hepatobiliary system [3, 11, 19] and of other organs [2].

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